Annals of Clinical and Analytical Medicine
Original Research

The effect of cerium oxide on erythrocyte deformability in ischemia-reperfusion injury in rats administered sevoflurane

Aydın Tunçay[1], Volkan Şıvgın[2], Faruk Metin Çomu[3], Ayşegül Küçük[4], Aycan Özdemirkan[2], Işın Güneş[5], Mustafa Arslan[2]
[1] Department of Cardiovascular Surgery, Medical Faculty, Erciyes University, Kayseri
[2] Department of Anesthesiology and Reanimation, Medical Faculty, Gazi University, Ankara
[3] Department of Physiology, Medical Faculty, Kırıkkale University, Kırıkkale
[4] Department of Physiology, Medical Faculty, Kutahya Health Sciences University, Kutahya
[5] Department of Anesthesiology and Reanimation, Medical Faculty, Erciyes University, Kayseri, Turkey

Abstract
Aim: Ischemia-reperfusion (IR) injury is a common problem in vascular surgery. Acute IR damage observed in the lower extremities, especially in aortic surgery, occurs following temporary cross-clamping of the abdominal aorta. Disruption in blood rheology disrupts microvascular blood flow, leading to exacerbation of microangiopathy. It is known that drugs used for anesthesia affect blood rheology, which is affected by many factors. Therefore, we aimed to investigate the effects of cerium oxide on erythrocyte deformability before sevoflurane anesthesia in rats with lower extremity IR.
Material and Methods: After approval by the ethics committee, 30 rats were randomly divided into 5 groups. Control (group C), IR (group IR), IR-cerium oxide (group IRCO), IR-sevoflurane (group IRS), IR-cerium oxide-sevoflurane (group IRCOS). Infrarenal abdominal aorta and atraumatic microvascular clamp were placed in IR groups 30 minutes after intraperitoneal cerium oxide was administered at a dose of 0.5 mg / kg. One hundred and twenty minutes later, the clamp was removed and reperfused for 120 minutes. Sevoflurane was applied at a rate of 2.3% at 4 L/min and 100% oxygen during IR for the minimum alveolar concentration to be 1 for rats. All rats were administered intraperitoneal ketamine (100 mg/kg) and euthanasia was performed by taking blood from the abdominal aorta. Erythrocytes were obtained from heparinized whole blood samples. Deformability measurements were made in erythrocyte suspensions in phosphate-buffered saline. A constant flow filtrometer system was used for the measurement of erythrocyte deformability and relative resistance was calculated.
Results: Erythrocyte deformability index was found to be significantly different between the groups (p=0.002). Compared to the control group, the erythrocyte deformability index was significantly higher in IR and IRS groups (p<0.0001, p=0.003, respectively). In the IRCO and IRCOS groups, the erythrocyte deformability index was found to decrease significantly compared to the IR group (p=0.008, p=0.025, respectively). The erythrocyte deformability index was similar in Group C and in the IRCO and IRCOS groups (p=0.453, p=0.120, respectively).
Discussion: We determined that cerium oxide administered intraperitoneally 30 minutes before ischemia in rats corrects the erythrocyte deformability deteriorated in IR-generated rats. We also found that cerium oxide had beneficial effects by reversing undesirable effects of IR. Further studies with larger volumes are required to support our promising results

Keywords
Ischemia-Reperfusion, Cerium Oxide, Sevoflurane, Erythrocyte Deformability, Rat

DOI: 10.4328/ACAM.21301 Received: 2022-07-04 Accepted: 2022-08-08 Published Online: 2022-08-11 Printed: 2022-12-01 Ann Clin Anal Med 2022;13(12):1305-1308
Corresponding Author: Mustafa Arslan, Department of Anesthesiology and Reanimation, Medical Faculty, Gazi University, 06510, Ankara, Turkey.
E-mail: mustarslan@gmail.com P: +90 533 422 85 77
Corresponding Author ORCID ID: https://orcid.org/0000-0003-4882-5063

Introduction

Ischemia- reperfusion injury (IR) in the lower extremity is a frequent and important clinical phenomenon. The period of reperfusion after an ischemic insult may paradoxically increase mortality and morbidity rates due to systemic complications. Local edema and muscle tissue necrosis are followed by systemic inflammatory response syndrome and multiple organ failure (kidney, respiratory and circulatory system, etc.) as reperfusion progresses [1,2].

Cerium oxide, an oxide of rare earth metal called cerium, is an important nanomaterial. It has a wide application area such as solar cells, fuel cells, gas sensors, oxygen pumps and is also used as a fuel additive [3].

The potential use of cerium oxide nanoparticles to stroke [4], sepsis [5], hepatic IR [6], and intestinal IR has been investigated in several studies [7].

Tatar et al. showed that cerium oxide has a potentially beneficial effect on erythrocyte deformability after IR. General anesthesia agents are known to affect cardiovascular functions and microcirculation dynamics [3].

General anesthesia agents are known to affect cardiovascular functions and microcirculation dynamics [8]. However, it is controversial whether these agents change plasma rheology and/or cause disruption of tissue perfusion. Changes in plasma viscosity are listed among the factors associated with anesthesia procedures responsible for disruption of tissue and organ perfusion [9,10]. Erythrocyte deformability and increased aggregation can be seen after surgical procedures under general anesthesia [10].

Capillary filtration coefficient decreases with sevoflurane. It has been shown that sevoflurane may have beneficial effects on microcirculation by reducing the extravasation of plasma into the interstitial space, and thus limiting tissue edema, compared to intravenous anesthetics such as propofol [11]. Sevoflurane may also have a protective effect against IR injury on endothelial cells [12].

Volatile anesthesia applied during general anesthesia increases peripheral perfusion. This correlation has been shown for anesthesia of sevoflurane on peripheral tissue flow [13].

In this study, we aimed to investigate the effects of cerium oxide applied on erythrocyte deformability before the sevoflurane anesthesia in rats with lower extremity IR.

Material and Methods

Animals and Experimental Protocol

This study was conducted in the Physiology Laboratory of Kirikkale University upon the consent of the Experimental Animals Ethics Committee of Gazi University. All procedures were performed according to the accepted standards of the Guidelines for the Care and Use of Laboratory Animals.

The subjects in our study were 24 Wistar Albino rats weighing between 200 and 250 g, which were nurtured in the same habitat. The subjects were kept under 20-21 oC within cycles of 12-hour daylight and 12-hour darkness. They were given free access to nutrition until 2 hours before the anesthesia procedure and randomly separated into five equal groups of 6 animals. Ketamine anesthesia was applied prior to midline laparotomy.

Control group (Group C): Midline laparotomy was the sole surgical procedure without any additional intervention. After 4 hours of follow-up, blood samples were collected and the subjects were sacrificed.

Ischemia-reperfusion group (Group IR): Midline laparotomy was done in a similar fashion. The infrarenal aorta was left clamped for 2 hours. After removing the clamp, reperfusion was established for another additional 2 hours. After 4 hours, blood samples were taken from the abdominal aorta and subjects were sacrificed

Ischemia-reperfusion group with cerium oxide (Group IRCO): After following the same steps as in the IR group, cerium oxide was administered (0.5 mg/kg) intraperitoneally 30 minutes before the ischemia period. After 4 hours, blood samples were collected from the abdominal aorta and subjects were sacrificed.

Ischemia-reperfusion group with sevoflurane (Group IRS): After following the same steps as in the IR group, anesthetic gas vaporizers were calibrated and set a minimum alveolar concentration (MAC) of 1 sevoflurane (2.3%). Anesthesia of rats was conducted in a transparent plastic box measuring 40X40X70 cm. The box, which allowed for observation of the rats, was connected to a half-open anesthesia machine with static hoses. Anesthetic gases were released into the container in 100% O2. Sevoflurane was administered at an inspiratory concentration of 2.3% at a rate of 4 L.min-1 in 100% O2 for 4 hours. At the end of 4 hours, blood samples were collected from the abdominal aorta and subjects were sacrificed.

Ischemia-reperfusion group with cerium oxide+ sevoflurane (Group IRCOS): After following the same steps as in IR group, cerium oxide was administered (0.5 mg/kg) intraperitoneally 30 minutes before the ischemia period. Anesthetic gas vaporizers were calibrated and set a MAC of 1 sevoflurane (2.3%), and the same procedures were performed. At the end of 4 hours, blood samples were collected from the abdominal aorta and subjects were sacrificed.

After anesthesia procedure, all rats were intraperitoneally injected with ketamine at a dose of 100 mg.kg-1. Heparinized total blood samples were used to prepare erythrocyte packs. Deformability measurements were carried out using erythrocyte suspensions with 5% HCT in phosphate-buffered saline buffer.

Deformability Measurements

Blood samples were taken very carefully and measurement process was as fast (the first 5 minutes) as possible to avoid hemolysis of erythrocytes. The collected blood was centrifuged at 1000 rpm for ten minutes. Serum and buffy coat on erythrocytes were removed. Isotonic PBS buffer was added to collapsing erythrocytes and centrifuged at 1000 rpm for ten minutes. The liquid on the upper surface was removed. Finally, pure red cell packs were obtained from the washing process, which was repeated three times. Erythrocytes packs were mixed with PBS buffer to generate a suspension with the value of 5% HCT. Those erythrocyte suspensions were used for the measurement of deformability. Collection and deformability measurements of erythrocytes were done at 22 Cº.

To measure the deformability of erythrocytes, the constant-current filtrometer system was used. Samples for measurement were prepared as 10 ml of erythrocytes suspension and PBS buffer. The flow rate was held constant at 1.5 ml/min with

an infusion pump. A 28 mm nucleoporin polycarbonate filter with a 5 µm pore diameter was used. Constant changes in pressure during the passage of erythrocytes through from the filter were detected by the pressure transducer and the data were transferred to a computer using an MP 30 data equation systems (Biopac Systems Inc, Commat, USA). The necessary calculations were performed with related computer programs by measuring pressure changes at various times. Pressure calibration of the system was performed each time before measuring the samples. Firstly buffer (PT) and then erythrocytes (PE) were passed through from the filtration system and pressure changes were measured. The relative refractory period value (Rrel) was calculated by relating the pressure value of erythrocytes suspension to pressure value of the buffer. An increase in Rrel as the deformability index was interpreted as a negative effect on the ability of erythrocytes to deform.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 17.0 program was used for statistical analysis. The significance of the difference in the mean erythrocyte deformability values was assessed using the Kruskal-Wallis test. The Bonferroni adjusted Mann-Whitney U test was used after the significant Kruskal-Wallis to determine which group differed from the other. The results were expressed as mean ± standard deviation (Mean ± SD). Statistical significance was set at a p- value<0.05.

Results

Erythrocyte deformability index was found to be significantly different between the groups (p=0.002). Compared to the control group, erythrocyte deformability index was significantly higher in IR and IRS groups (p<0.0001, p=0.003, respectively). In the IRCO and IRCOS groups, the erythrocyte deformability index was found to decrease significantly compared to the IR group (p=0.008, p=0.025, respectively). The erythrocyte deformability index was similar in Group C and in the group IRCO and IRCOS groups (p=0.453, p=0.120, respectively), (Figure 1).

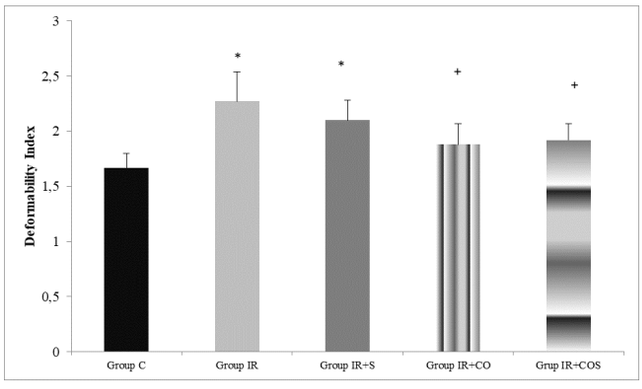

Figure 1. Erythrocyte deformability values of the groups. Each bar represents the mean ± standard deviation.
* p<0.05 compared to the Group C; + p< 0.05 compared to the Group IR

Discussion

There are numerous studies showing a drastic increase in oxidative stress upon reperfusion [14,15].

Effective blood flow in microcirculation is necessary to maintain tissue perfusion. Rheological measurements performed in patients undergoing major elective surgery revealed increased blood viscosity, fibrinogen and erythrocyte aggregation due to decreased erythrocyte deformability, decreased blood flow in microcirculation and therefore oxygen delivery to tissues. It is well known that anesthetic agents are among numerous factors affecting blood rheology [16].

Erythrocytes deform when passing through capillaries smaller than their diameter. Erythrocyte deformability is determined by many factors, including the ratio of surface area to volume, the phospholipid composition of the erythrocyte cell membrane and the viscosity of the intracellular fluid. Reduced erythrocyte deformability causes disruption of tissue perfusion in peripheral tissues [17].

Erythrocyte membranes are vulnerable to lipid peroxidation due to the lipid components of their membranes. Lipid peroxidation has negative effects on the deformability of erythrocytes [18].

Aydogan et al [19] showed the negative effects of sevoflurane on the deformability in old rats.

Comu et al [20] found that neither desflurane nor sevoflurane had a negative effect on erythrocyte deformability in diabetic male rats.

Aydın et al [21] showed that neither desflurane nor sevoflurane had a negative effect on erythrocyte deformability in infrarenal aorta of diabetic rats undergoing IR.

It was shown that cerium oxide nanoparticles remain in the circulation for a short period of time such as t1/2 of 7.5 min. upon intravenous injection [22]. It has been shown that oxidative stress drastically increases upon reperfusion [14,15] and the administration of cerium oxide nanoparticles one hour prior to ischemia would result in bioaccumulation of cerium oxide nanoparticles in liver and scavenge ROS that have been generated during reperfusion

Kotsuruba et al [23] used 0.1 mg/kg of nanocerium per os for 14 days in old rats, which fully restored the resistance of erythrocytes to acid hemolysis by ROS and RNS in both plasma and erythrocytes reduction. Nanocerium decreased the erythrocytes and, conversely, significantly increased the plasma's pools of H2S.

Conclusion

The results of this study clearly demonstrated that erythrocyte deformability is significantly altered in experimental infrarenal aorta IR injury and sevoflurane inhaled rats. This might lead to further problems in microcirculation. Thus, measurement of erythrocyte deformability might have an important impact on the follow-up for IR injury. Additionally, we were able to document the potential beneficial effect of cerium oxide on maintaining erythrocyte deformability in infrarenal aorta of rats undergoing IR. Other aspects of these findings, including clinical significance and practical applications, merit further experimental and clinical investigation.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: *None*

Conflict of interest
None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References
1. Duru S, Koca U, Oztekin S, Olguner C, Kar A, Coker C, et al. Antithrombin III pretreatment reduces neutrophil recruitment into the lung and skeletal muscle tissues in the rat model of bilateral lower limb ischemia and reperfusion: a pilot study. Acta Anaesthesiol Scand. 2005;49(8):1142-8.
2. Turchányi B, Tóth B, Rácz I, Vendégh Z, Furész J, Hamar J. Ischemia reperfusion injury of the skeletal muscle after selective deafferentation. Physiol Res. 2005;54(1):25-32.
3. Tatar T, Polat Y, Comu FM, Kartal H, Arslan M, Kucuk A. Effect of cerium oxide on erythrocyte deformability in rat lower extremity ischemia reperfusion injury. Bratisl Lek Listy. 2018;119(7):441-3.
4. Estevez AY, Pritchard S, Harper K, Aston JW, Lynch A, Lucky JJ, et al. Neuroprotective mechanisms of cerium oxide nanoparticles in a mouse hippocampal brain slice model of ischemia. Free Radic Biol Med. 2011;51(6):1155-63.
5. Manne ND, Arvapalli R, Nepal N, Shokuhfar T, Rice KM, Asano S, et al. Cerium oxide nanoparticles attenuate acute kidney injury induced by intra-abdominal infection in Sprague-Dawley rats. J Nanobiotechnology. 2015;13:75.
6. Manne NDPK, Arvapalli R, Graffeo VA, Bandarupalli VVK, Shokuhfar T, Patel S, et al. Prophylactic Treatment with Cerium Oxide Nanoparticles Attenuate Hepatic Ischemia Reperfusion Injury in Sprague Dawley Rats. Cell Physiol Biochem. 2017;42(5):1837-46.
7. Gubernatorova EO, Liu X, Othman A, Muraoka WT, Koroleva EP, Andreescu S, et al. Europium-Doped Cerium Oxide Nanoparticles Limit Reactive Oxygen Species Formation and Ameliorate Intestinal Ischemia-Reperfusion Injury. Adv Healthc Mater. 2017;6(14).
8. Erdogan C, Erdem A, Akıncı SB, Dikmenoglu N, Basgül E, Balkancı D, et al. The effects of midazolam on erythrocyte deformability and plasma viscosity in rats. Anestezi Dergisi 2005;13:205-8.
9. Cho AR, Lee HJ, Kim HJ, Do W, Jeon S, Baek SH, et al. Microvascular Reactivity Measured by Dynamic Near-infrared Spectroscopy Following Induction of General Anesthesia in Healthy Patients: Observation of Age-related Change. Int J Med Sci. 2021;18(5):1096-103.
10. Cui X, Ma L, Wei J. A clinical study on the effects of dexmedetomidine and propofol on erythrocyte deformability during anaesthesia. Trop J Pharm Res. 2022; 21(2): 333-9.
11. Bruegger D, Bauer A, Finsterer U, Bernasconi P, Kreimeier U, Christ F. Microvascular changes during anesthesia: sevoflurane compared with propofol. Acta Anaesthesiol Scand. 2002;46(5):481-7.
12. Annecke T, Chappell D, Chen C, Jacob M, Welsch U, Sommerhoff CP, et al. Sevoflurane preserves the endothelial glycocalyx against ischaemia-reperfusion injury. Br J Anaesth. 2010;104(4):414-21.
13. Hager H, Reddy D, Kurz A. Perfusion Index-a valuable tool to assess changes in peripheral perfusion caused by sevoflurane? Anesthesiology 2003;99: A593.
14. Montalvo-Jave EE, Escalante-Tattersfield T, Ortega-Salgado JA, Piña E, Geller DA. Factors in the pathophysiology of the liver ischemia-reperfusion injury. J Surg Res. 2008;147(1):153-9.
15. Xu Z, Yu J, Wu J, Qi F, Wang H, Wang Z, et al. The Effects of Two Anesthetics, Propofol and Sevoflurane, on Liver Ischemia/Reperfusion Injury. Cell Physiol Biochem. 2016;38(4):1631-42.
16. Ramakrishnan S, Grebe R, Singh M, Schmid-Schönbein H. Influence of local anaesthetics on the aggregation and deformability of erythrocytes. Clin Hemorheol Microcirc. 1999;20(1):21-6.
17. Unal FA, Erolcay H. Effect of magnesium sulphate infusion on blood rheology during gynecologic oncology surgery. Göztepe Tıp Dergisi. 2012; 27(4):174-81.
18. Tavazzi B, Di Pierro D, Amorini AM, Fazzina G, Tuttobene M, Giardina B, et al. Energy metabolism and lipid peroxidation of human erythrocytes as a function of increased oxidative stress. Eur J Biochem. 2000;267(3):684-9.
19. Aydoğan S, Yerer MB, Comu FM, Arslan M, Güneş-Ekinci I, Unal Y, et al. The influence of sevoflurane anesthesia on the rat red blood cell deformability. Clin Hemorheol Microcirc. 2006;35(1-2):297-300.
20. Comu FM, Şıvgın V, Özköse Z, Arslan M. Comparative effects of sevoflurane and desflurane on erythrocyte deformability in streptozotocin-induced diabetic rats. British Journal of Medicine & Medical Research. 2014;4 (22):3954-62.
21. Aydın ME, Erbatur ME, Çomu FM, Arslan M. Effect of sevoflurane and desflurane on erythrocyte deformability during ischaemia-reperfusion injury of lower extremity in diabetic rats. Int J Anesthetic Anesthesiol. 2015;2:026.
22. Yokel RA, Hussain S, Garantziotis S, Demokritou P, Castranova V, Cassee FR. The Yin: An adverse health perspective of nanoceria: uptake, distribution, accumulation, and mechanisms of its toxicity. Environ Sci Nano. 2014;1(5):406-28.
23. Kotsuruba AV, Kopjak BS, Sagach VF, Spivak NJ. Nanocerium restores the erythrocytes stability to acid hemolysis by inhibition of oxygen and nitrogen reactive species in old rats. Fiziol Zh (1994). 2015;61(1):3-9.

How to cite this article:
Aydın Tunçay, Volkan Şıvgın, Faruk Metin Çomu, Ayşegül Küçük, Aycan Özdemirkan, Işın Güneş, Mustafa Arslan. The effect of cerium oxide on erythrocyte deformability in ischemia- reperfusion injury in rats administered sevoflurane. Ann Clin Anal Med 2022;13(12):1305-1308